AN EVALUATION OF THE SIGNIFICANCE OF MUMPS HEMAGGLUTINATION INHIBITION TITERS IN NORMAL POPULATIONS

WILLIAM JOHN HOUGHTON

1964

An Evaluation of the Signifigance of Mumps Hemagglutination Inhibition Titers in Normal Populations

by

William John Houghton

A thesis presented to the faculty
of the Yale University School of Medicine
in partial fulfillment of the requirements
for the Degree of Doctor of Medicine

Department of Epidemiology and Public Health

1964

ACKNOWLEDGEMENT

To Dr. Francis L. Black and Dr. G.-D. Hsiung, without whose help this thesis would not exist.

TABLE OF CONTENTS

INTRODUCTION

Serological tests for mumps.

In a disease like mumps, in which a large percentage of the infections are inapparent (1), epidemiological studies are most reliable when they classify cases according to the presence or absence of a stable, specific antibody, rather than according to history.

Tests available. The discovery by Levens and Enders in 1945 (2) that convalescent sera inhibited hemagglutination by the mumps virus led to the hemagglutination inhibition (HI) test, which subsequently proved to be the most accurate index of mumps immunity presently available. For years, the HI test has been considered to be more sensitive than the complement fixation (CF) test in detecting recent infections (3,4), to remain positive longer (5), and to be as reliable as the neutralization (N) or skin hypersensitivity tests in detecting previous infections (6). Following the Salk modifications in HI technique, nonspecific inhibitors in normal sera have not been a problem (3,4).

Signifigance of tests. Retrospective studies of mumps immunity, to gain an idea of the place of mumps in the broad spectrum of disease, have seldom been performed. Two reasons for this are the frequency of inapparent mumps infections (rendering histories unreliable), and uncertainty about the validity of serological techniques in detecting previous infections. It is uncertain how long mumps antibodies last. It is thought that CF antibodies last about 6 months, neutralizing antibodies six years or longer (6), and HI antibodies

an unknown period, probably at least as long as neutralizing antibodies. In contrast to the varying persistence of these antibodies, immunity to reinfection with mumps is thought to be lifelong. The accuracy of all these serological tests depends greatly on the skill with which they are performed. Maris reported much longer persistence of complement-fixing antibodies (28).

Cross reactions with other viruses.

Recently, the reliability of the HI test in the diagnosis of mumps has been further questioned because studies have indicated that other myxoviruses, especially the parainfluenza viruses, can stimulate mumps antibodies.

Nature of viruses related to mumps. All myxoviruses share certain physical properties (7,8). The mumps virus is believed to contain an antigen system which gives rise to a number of probably distinct antibodies (complement fixing, hemagglutinating, and neutralizing antibodies) (9). A similar situation may obtain for the other myxoviruses. The antigens in different myxoviruses may be enough alike to produce antibodies so similar that they cross react.

Extent of cross reactions. This is pertinent to the serology of mumps. If infections with another myxovirus, e.g. parainfluenza, gave rise to mumps antibodies (and this is reported to occur) this would produce false positive mumps diagnoses. This might create an erroneously high percentage of positive tests in non-immune persons. From serology and history alone these could not be distinguished from

inapparent mumps infections.

The literature abounds with conflicting information about the directions of cross reactions (some shown in Figure 6). For example, following Chanock (7), Lennette (11) has shown that mumps produces a rise in parainfluenza titers. Cross reactions between influenza viruses or adenoviruses and mumps were not detected. In his study, isolation of viruses was not attempted. Diagnoses were made by the clinical picture or serology. The argument that mumps produces parainfluenza antibodies because both mumps and parainfluenza antibodies are present is a circular one. The question is whether the parainfluenza antibody is similar enough to the mumps antibody to render the mumps diagnosis incorrect.

The recent work of Hsiung (10), however, in which 7 of 10 cases from which parainfluenza or DA virus was isolated showed signficant rises in mumps antibodies, is strong evidence that parainfluenza does produce mumps antibodies, at least temporarily.

The situation is made more complex by the recent suggestion that parainfluenza viruses may play a role in so-called "second cases of mumps" (12). Parainfluenza 1 was isolated, and the titers of neutralizing antibodies to that agent rose, in a patient with clinical mumps who showed no rise in mumps HI titer. (This suggestion will be discussed later.)

Epidemiology of mumps.

Pattern in developed areas. Several important characteristics of the epidemiology of mumps might be mentioned. Mumps is an acute

infectious disease with distinctive features in typical cases. Its mode of transmission is respiratory. In developed areas, between 60 and 90% of the population have had experience with mumps, by history and/or serology (1,28). In about 30% of the cases, infection was inapparent; though antibodies to mumps are present, the subject gives a negative history (1,6). The communicability of mumps is less than that of, for example, measles. Evidence for this is the considerable percentage of susceptibles of all ages in many urban areas, and the existence of whole populations which are susceptible.

Pattern in isolated areas. R. Philip has recently described an outbreak of mumps on the isolated island of St. Lawrence (13). Eight-eight percent of the population contracted mumps, as diagnosed by clinical picture, and often by a significant rise of CF antibodies. As Philip suggested, study of an isolated population allows an investigator to abstract the "inherent infectiousness" of an agent, and "the inherent susceptibility" of a population. If only one of two related agents occurs in an isolated area, the effect of that particular agent can be studied without any chance of interference by the other agent.

Theories of partial immunity to mumps in adults. The "susceptibility" of a population varies most obviously with its past experience with an infectious agent. But even within a population which is highly susceptible and has no easily recognized (historical or humoral) past experience of an agent, there may be differences

in "inherent susceptibility". For example, Philip (13) found that in the St. Lawrence Island mumps epidemic, adults over 35 had the lowest attack rate, the lowest morbidity, the highest percentage of inapparent infections, and the highest antibody rises following clinical infections. This occurred despite the fact that prior to the epidemic adults showed no significant mumps titers (but only CF antibodies were measured) and there was no history of a mumps epidemic for at least 50 years.

Possible explanations for this are: a decline in "inherent susceptibility" with age, i.e., an unknown physiological mechanism. Or secondly, the adults could have had experience with mumps in the remote past. Philip considers this unlikely. Previous mumps experience would probably have produced solid immunity, but the reaction of those over 35 years old was a mixture of partial immunity and sensitivity. Or thirdly, the adults could have obtained some degree of immunity by forming cross-reacting antibodies—experience with "Agent X" giving them some immunity but no persistent, detectable antibodies against mumps. Then the present epidemic could have boosted their mumps titers to higher levels than were seen in the younger age group. This fits with the fact that, in other populations, the incidence of heterologous cross reactions is higher in older people than in younger (Lennette's report on mumps (11)). It would be logical to suspect that "Agent X" was a member of the myxovirus family.

Epidemiology of the parainfluenza viruses.

Parainfluenza infections are associated with pneumonitis, laryngotracheitis, and croup, entities far less distinctive than parotitis. These infections occur most often in infants and preschool children (21,22) though some have definitely been diagnosed in adults. Parainfluenza infections are transmitted by the respiratory route. Some of the parainfluenza viruses are highly communicable. Evidence for this are the abrupt outbreaks reported among young children and the wide distribution of parainfluenza antibodies in adults. Lennette (11) found that 80% of his small groups of recruits had HI titers of greater than 1:32 against parainfluenza 1 and 3. Chanock (23) found that only 11% of young (<5 years) children had titers of N antibodies sufficient to provide immunity (>4). In the same young group 61% had titers of neutralizing (N) antibodies against parainfluenza 3 of greater than 1:32. Hsiung (10) found that in New Haven by 6-10 years of age 90% had significant HI titers (20 or >) to parainfluenza 3, and 50% to parainfluenza 1. La Placa (24) in a study covering samples from Colorado, Italy, and India, found an almost identical pattern in each country. With differences of only a few percentage points, about 40% of the children (<10 years) had positive serologies (mostly HI) against parainfluenza 2, and 69% against parainfluenza 3. By adulthood, 84% were parainfluenza 1 positive, 76% Sendai positive, 25% parainfluenza 2 positive, and 97-100% parainfluenza 3 positive. None of his communities were geographically isolated. The sume of this

experience seems to be that following intermediate titers in childhood, adults from many parts of the world have high parainfluenza 1 and 3 antibodies; fewer have Sendai and parainfluenza 2 antibodies.

In all of these studies HI and neutralization tests are considered to be of nearly equal value (23,24). The obscurity of the disease, the absence of histories, the necessity of isolates, and the possibility of second or double infections have combined to obviate discussions of the reliability of the serological tests in diagnosing individual old infections.

PURPOSE OF PRESENT STUDY

In the present paper brief surveys of mumps are made in several isolated populations. Observations are made on the histories, and mumps antibodies as measured by the HI technique. The reliability of mumps HI titers is examined with special reference to duration of antibody in the serum and the possible production of mumps false positive tests by parainfluenza cross reactions.

MATERIALS AND METHODS

The sera.

The collection of specimens. After venipuncture, the blood was allowed to clot in non-heparinized tubes. The tubes were centrifuged to separate clot from serum. Sera were stored at -20°C from the time of arrival at the field laboratory until testing.

The sources of sera. Sera were available from Baffinland, Alaska, Tahiti, and Iceland.

Many of the Baffinland and Alaskan sera were drawn from Eskimos on the mainland of Canada or on the Arctic islands (in 1949 and the early 1950's). These people lived as nomads, gathered in small villages (under 200), or rarely formed villages as large as Point Barrow (population 750). Outside contacts were limited to a few freighters and rare airplane visits. Thus, they were semi-isolated in the villages, or strictly isolated. Villages were periodically swept by severe epidemics of diseases such as pertussis, measles, or influenza. Common diseases such as mumps were sometimes absent from a population for decades. The Alaskan sera were collected by Dr. J.R.Paul, and the Baffinland sera were collected by Dr. L. Greenberg (15,16).

The Tahitian sera were collected by Dr. D.M. Horstman from Polynesian and Chinese natives on the islands of Tahiti and Raiatea (14). Although Tahiti had a population of 30,500, and some areas were crowded, there was relatively little contact of natives with the outside world. Freighters brought few tourists to the islands.

DISTRIBUTION OF
AGES IN ISOLATED POPULATIONS

AGES	ALASKA	BAFFINLAND	TAHITI 1951	TAHITI 1959
0-10 years old	15 Seka	0	72	43
10-20	31	3	84	39
20-30	23	10	17	13
30-40	18	6	5	5
40-50	6	4	2	1
>50	5	2	0	1

TABLE I

In 1950, the first measles epidemic in 22 years occurred. Paired sera, with an 8-year interval, were available from about 50 residents of Tahiti.

The Iceland sera were collected in 1962 by Dr. F.L. Black, partly from crowded areas near Reykjavik, but mostly from isolated rural areas. Rural areas were isolated enough so that sera could be selected for measles susceptibility as a part of another study (30,31).

The ages of subjects. The ages of the persons from whom sera were drawn in the isolated areas is shown in Table 1.

The test for mumps.

The hemagglutination inhibition (HI) test for mumps was carried out using the technique of Robbins (4) who employed the modifications of Salk (18) to eliminate nonspecific inhibitors in normal serum, with the exception that Sykes monkey red blood cells were used instead of chicken red blood cells.

The treatment of sera. To 0.1 cc of serum, 0.9 cc of a kaolin suspension was added (10 parts 25% kaolin in PBS plus 8 parts of PBS). This suspension was allowed to stand 20 minutes, and was then centrifuged at 1000 rpm for 10 minutes. The precipitate was discarded. To the supernatant .025 cc of packed Sykes RBC's was added, and the solution was held for 1 hour at 4°C. Following 10 minutes of centrifugation at 1000 rpm, the RBC button was discarded, and the extracted serum was stored in the refrigerator.

The antigen. Mumps viral antigen prepared by Microbiological Associates and diluted 1:16 was sonicated for 5 minutes to disperse

aggregates and increase agglutinating activity. Two tenths cc of antigen was diluted serially in 0.2 cc PBS, another 0.2 cc of PBS was added to each tube in the row (in place of serum), and 0.2 cc of ~~1%~~ a 1% solution of Sykes red blood cells was added to each tube. The cells were allowed to settle at room temperature and agglutination was read according to the description of Salk. A hemagglutinating unit was defined as the maximum dilution of virus producing complete agglutination.

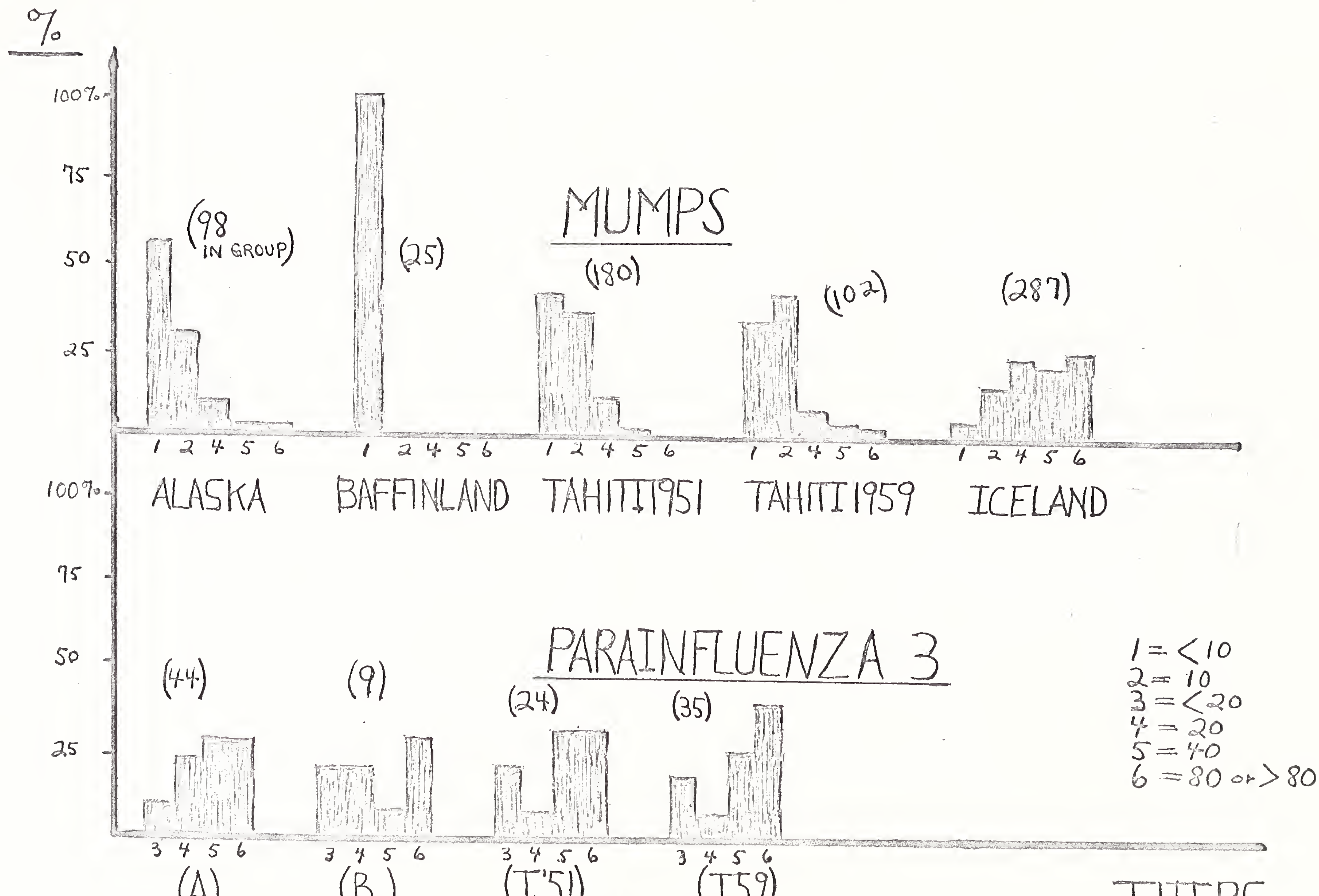
The titration. In the titration of serum, 0.2 cc of serum was diluted serially in 0.2 cc of PBS (usually out to 1:320). The mumps antigen was diluted so that 1 cc contained 4 hemagglutinating units (dilution seldom varied more than twofold, because fresh antigen was used), and 0.2 cc of this strength antigen was added to each dilution of serum. The mixture of virus and serum was allowed to incubate for 1 hour at room temperature. Two tenths cc of a 1% suspension of Sykes RBC's was added to each tube, and HI was read (Salk) on first settling. The reciprocal of the highest dilution showing HI was called the HI titer.

The test for parainfluenza 1 and 3.

Parainfluenza titers were measured by the HI technique of Hsiung (10,19).

The treatment of sera. Sera were cleared of nonspecific inhibitors either by 0.9 M potassium periodate or by 25% kaolin treatment.

The antigen. Parainfluenza 1 and 3 virus were grown from specimens donated by Dr. G.D. Hsiung in monkey kidney culture with HLS (Hanks-lactalbumin-serum) medium. Anti-SV-5 serum was added



%
100%
75
50
25
MUMPS
(98 IN GROUP)
(25)
(180)
(102)
(287)
1 2 4 5 6
ALASKA
BAFFINLAND
TAHITI 1951
TAHITI 1959
ICELAND
PARAINFLUENZA 3
(44)
(9)
(24)
(35)
3 4 5 6
(A.)
(B.)
(T.'51)
(T.59)
1 = <10
2 = 10
3 = <20
4 = 20
5 = 40
6 = 80 or >80
TITERS

FIGURE 1

to prevent interference by that agent. The suparnatant was titrated for hemagglutinating activity each day it was used, and the parainfluenza 3 virus was typed with a specific antiserum. No antiserum was available to type the parainfluenza 1 virus.

The titration. HI tests were performed in polyethylene panels or 10 mm tubes. Four units of viral hemagglutinin were added to each serum dilution, and a 0.5% suspension of guinea pig erythrocytes was used as the indicator system.

RESULTS

Mumps antibody surveys.

Isolated areas. The sera titrated from Baffinland, Alaska, and Tahiti appear to be relatively lacking in mumps antibodies. A total of 406 sera were tested. A total of 19 or 5% had mumps antibody titers of 40 or greater (64 or 15% had titers of 20 or greater).

The distribution of titers varied little among these 3 populations as shown in Figure 1. There were no titers of 40 or more out of 25 sera from Baffinland. There were 4 titers of 40 or more among 98 sera from Alaska (4%). There were 15 titers of 40 or more among 283 sera from Tahiti (5%).

Because there were so few high titers, their distribution according to age is not presented; high titers were scattered through all the age groups shown in Table 1.

The overall incidence of HI antibodies of 4% among Baffinland, Tahitian, and Alaskan sera suggests it is an antibody that

is maintained only by repeated exposures which are relatively few in these areas.

Changes in 8-year interval. The Tahitian sera were collected at two different times, first in 1951, and later in 1959. Of the 180 1951 sera, only 6 (or 3%) had titers of 40 or more. Of the 103 1959 sera 9 (or 8%) had titers of 40 or more. Possible explanations for the difference in these titers are: loss of 1951 antibody during cold storage (though other antibodies were not lost), a higher incidence of mumps after 1951 than before, or a higher incidence of cross reactions in 1959.

Forty-five of the inhabitants of Tahiti were represented by sera drawn both in 1951 and 1959. In this group one of the 45 sera drawn in 1951 had a titer of 40 or more (2%); and 6 of the 45 sera drawn in 1959 (13%) had titers of 40 or more. The one high titer in 1951 declined by 1959 from a titer of 40 to one of 10. The 6 high titers of 1959 had climbed from titers of 10-20 in 1951 to titers of 40-160 in 1959. The 6 persons with titer rises were 11, 19, 20, 33 and 35 years old in 1959. This is like the age range of the population sampled, as shown in Table 1.

Correlations with parainfluenza serology are reported later in the paper.

Iceland. The survey of mumps antibodies in Iceland shows the different patterns of endemic infection (with repeated epidemics).

Two hundred and sixty-two sera from 11 geographical areas were examined. Overall, the average titer of mumps HI antibodies was 35.

MUMPS IN ICELAND

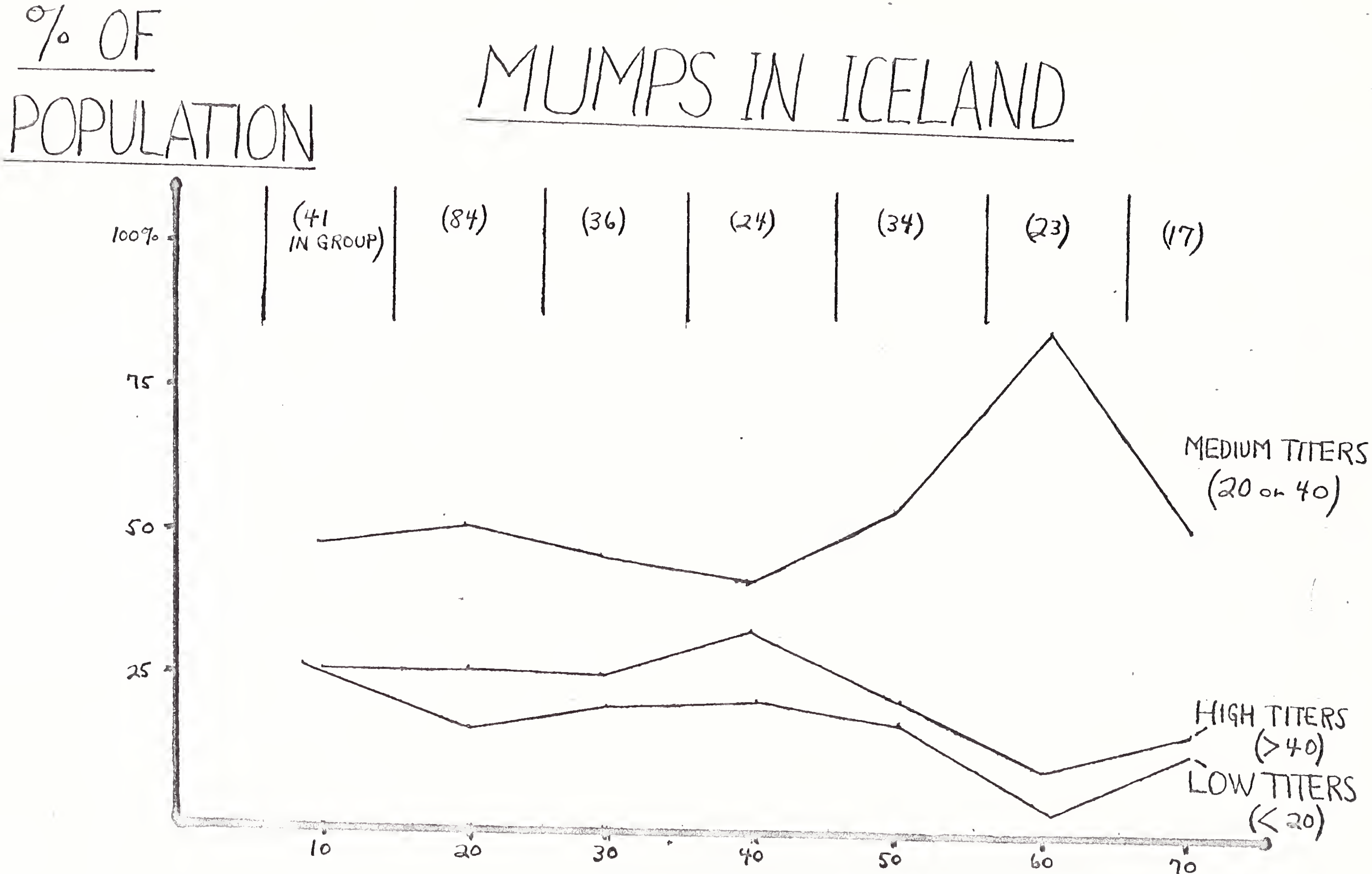


FIGURE 2

The average did not vary appreciably from district to district.

Of the 262 sera, 134 or 51% had titers of 40 or more (22.6 or 86%, titers of 20 or more).

The distribution of titers is shown in Figure 1. Unlike Tahiti, Baffinland, and Alaska, in Iceland there were many high and few low titers.

<u>Correlation with age</u>. The distribution of titers according to age is shown in Figure 2, where the percentage of the population with high, medium or low titers is plotted against age. The numbers in each 10-year group are in parentheses at the top of the column. Too few sera from children under 10 years old were available to give an accurate idea of mumps antibodies in that group. At age 10, 29% of the population have high tiers (>40); 43% have medium titers (20 or 40); and 28% have low titers (<20). This pattern persists with little change until age 40. At 40 several trends begin which culminate at 60; high and low titers decrease, and moderate titers climb. At age 60, 8% of the population have high titers, 87% have moderate titers, and 5% have low titers. The group over 60 years old, like that under 10, contains too few specimens to be significant. It must be realized that Figure 2 represents a cross-section of the population at one point in time, not the picture of mumps antibodies changing through time.

Data published in the Journal of Public Health in Iceland (20) show that epidemics have occurred with increasing frequency since World War II. Hence the age pattern is not a reflection of continued exposure to the same influences, but may reflect primarily

MUMPS IN ICELAND

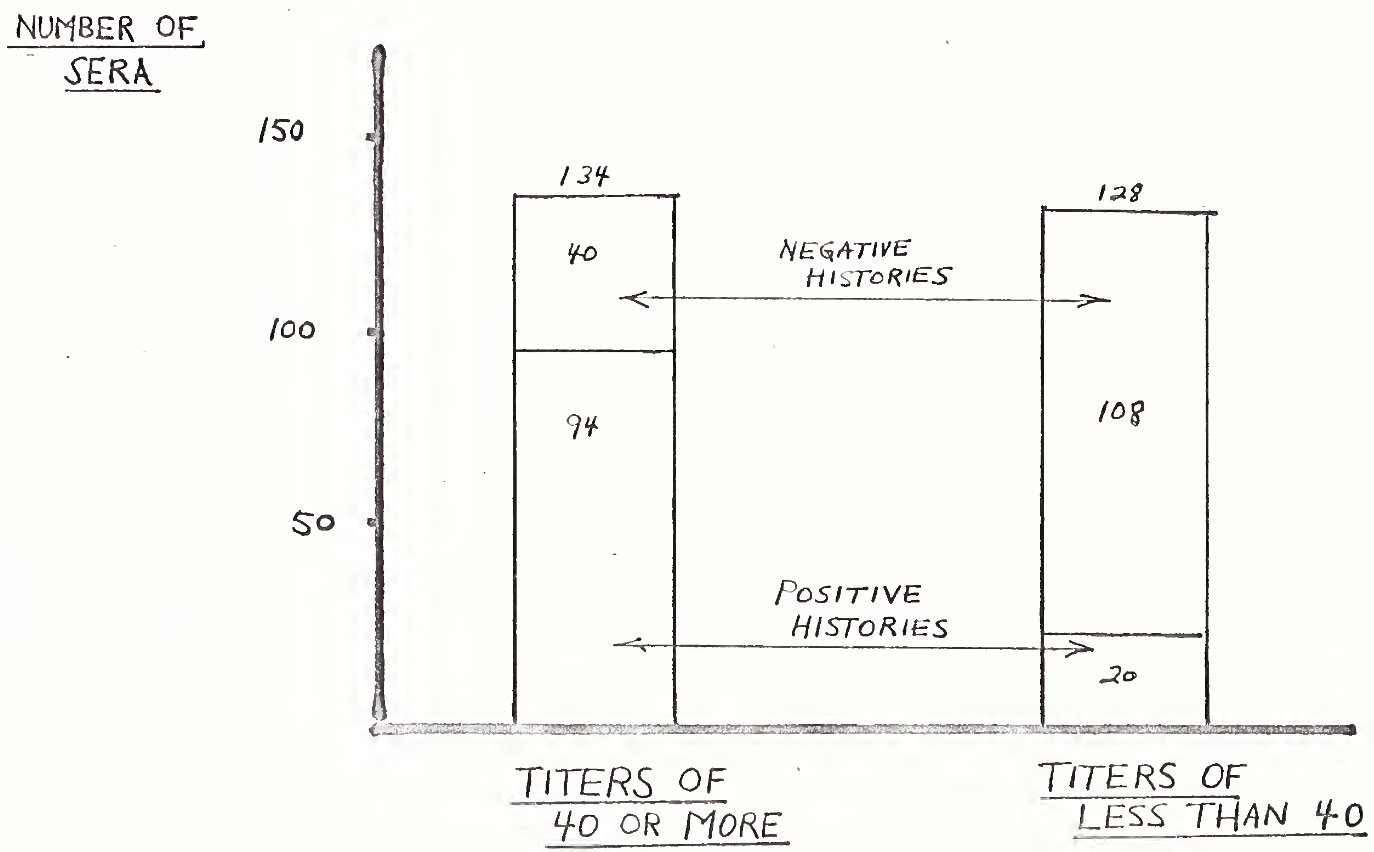


FIGURE 3

MUMPS IN ICELAND

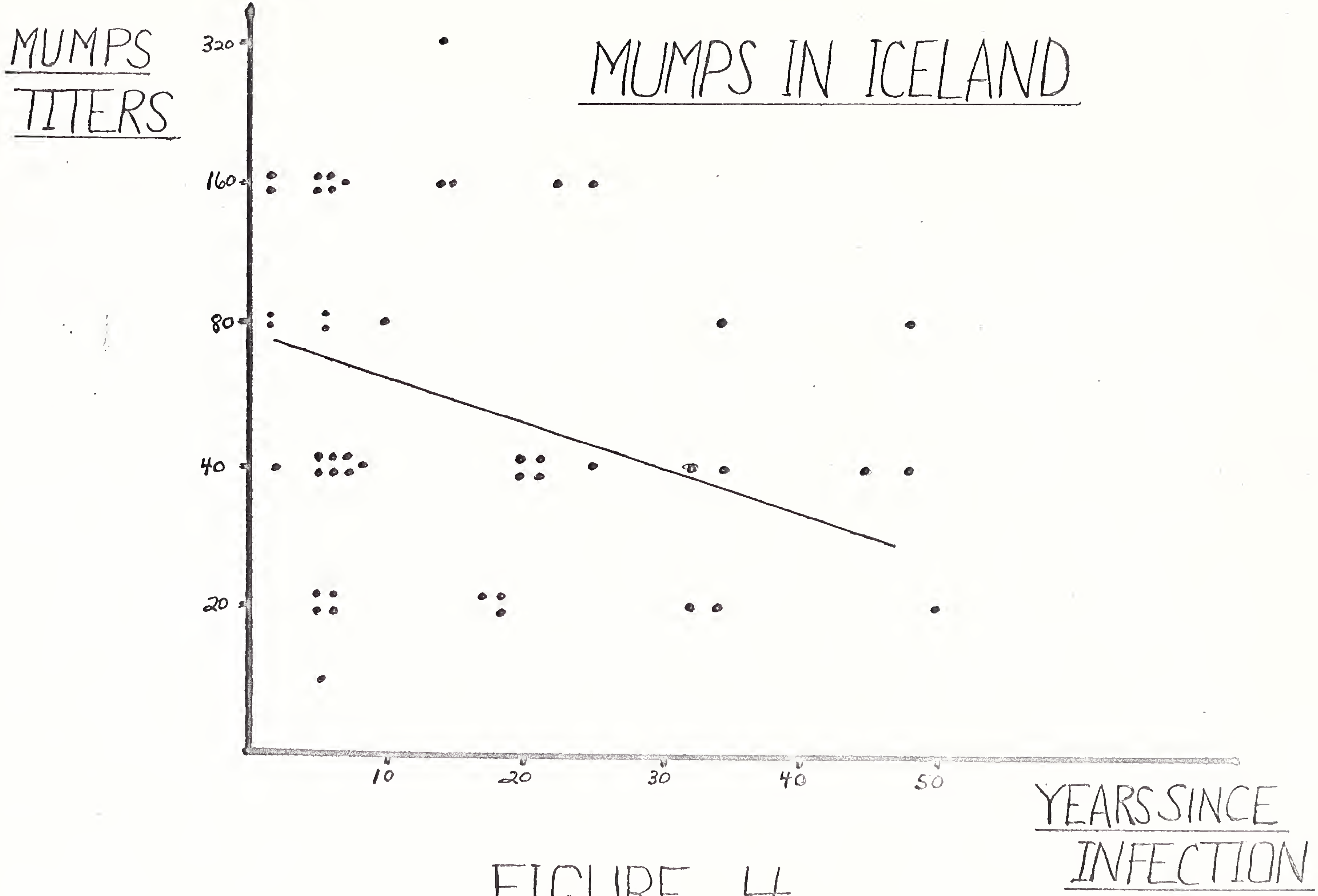


FIGURE 4

recent history.

Correlation with history. Two hundred and thirty-four of the Iceland inhabitants gave a mumps history. One hundred and five said they had had mumps; 129 said they had not; 6 were doubtful; and 25 gave no reply.

Of the 134 sera with titers of 40 or greater, 40 gave negative histories (see Figure 3). Thus, 30% acquired mumps antibodies without remembered disease. This 30% is fairly close to the usual figure for inapparent mumps infections (1,6).

There was another group composed of 20 people with positive histories and mumps titers of less than ~~20~~ 40 (see Figure 3). This was 17% of the positive histories recorded in the present sample. Other studies (see below) have reported negative or insignificant titers in up to 30% of persons with positive histories. Possible explanations of this group are unreliable histories, or decay of mumps antibodies. The first explanation would not have been considered significant until Bloom suggested (12) that there might be several viruses causing mumps.

Decay of mumps antibody *in vivo*. Decay of mumps antibodies was hard to estimate because paired sera were not available (except in the Tahitian sera where antibodies increased between 1951 and 1959). A rough indication of the rate of decay can be obtained from the data presented in Figure 4. The mean line drawn on this figure is only an approximation.

Forty-nine of the Icelanders gave information on the interval since they were infected with mumps. As the graph shows, most of

these people (33) had had mumps within 20 years. The group infected between 10 and 20 years previously had an average titer nearly as high as the group infected within the last 10 years. In the groups with infections more than 20 years previously, the number of individuals is admittedly small, but the average titers and percentages of titers of 40 or more held up, though very high titers do not appear. Only one person had a titer of less than 20 after a recent infection, and in this case the infection had occurred 6 years previous to testing. This Figure suggests that mumps HI antibodies, at least after a case severe enough to be remembered, persist in good titer for at least 20 years.

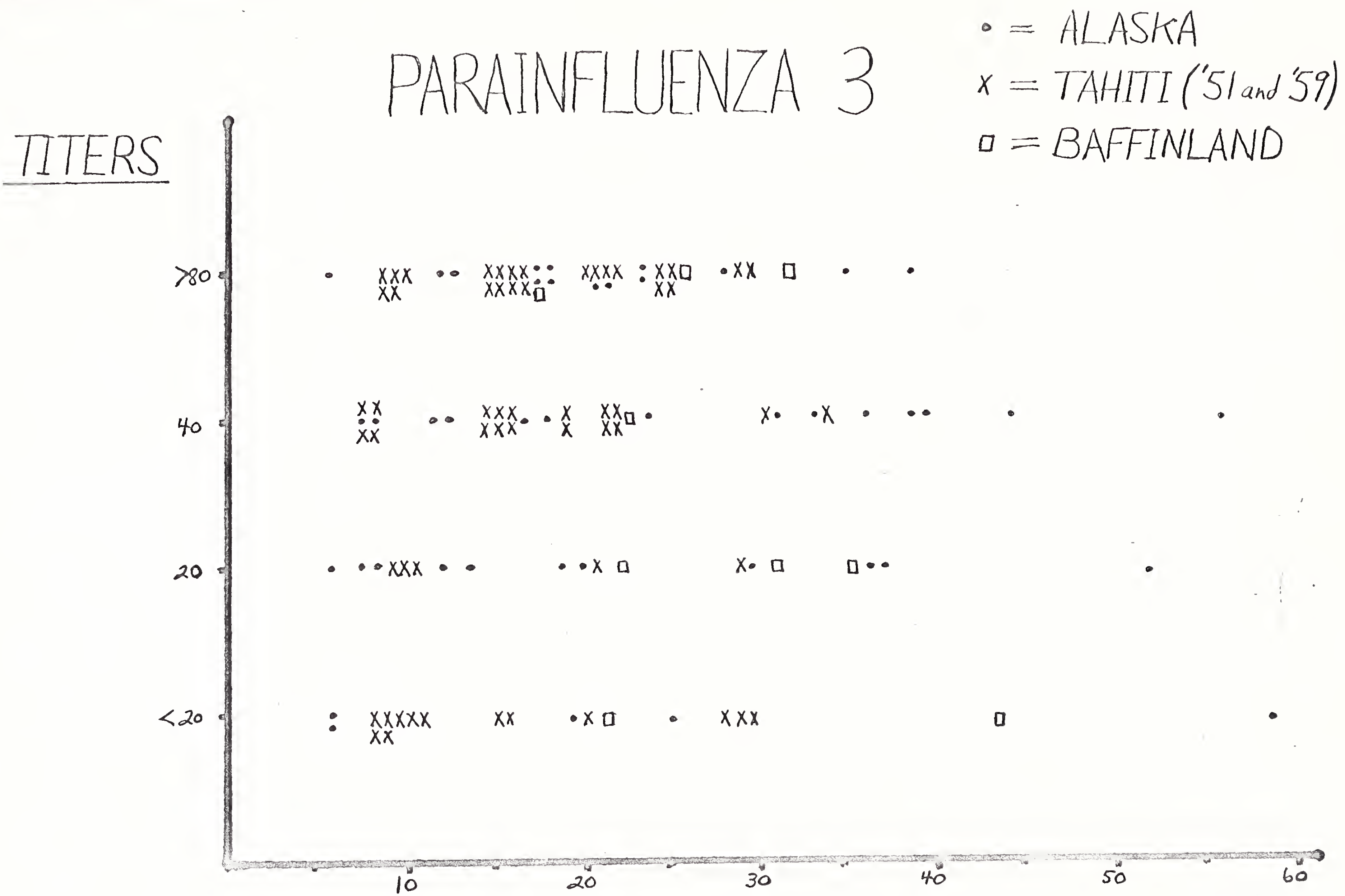
Parainfluenza 1 and 3 antibody surveys.

The titers of parainfluenza 1 and parainfluenza 3 antibodies were measured among inhabitants of Alaska, Baffinland and Tahiti, both the 1951 and 1959 specimens.

Parainfluenza 1. Of 31 sera tested from Alaska and 49 sera from Tahiti, all the titers of parainfluenza 1 antibodies were less than 20. Most of the parainfuenza 1 titers in the isolated populations were run with dilutions beginning at 1:20. Perhaps a number of 1:10 titers were missed, which some investigators would consider positive. Otherwise parainfluenza 1 may be scarce in these populations.

Parainfluenza 3. The test for parainfluenza 3 antibodies detected a high prevalence in all three populations. These results are shown in Figure 1.

Among the Alaskan sera 39 out of 44 tested (88%) had a titer



PARAINFLUENZA 3
• = ALASKA
X = TAHITI ('51 and '59)
□ = BAFFINLAND
TITERS
>80
40
20
<20
10
20
30
40
50
60
AGE

FIGURE 5

of parainfluenza 3 antibodies of 20 or greater.

Forty-six out of 59 Tahitian sera (77%) had parainfluenza 3 titers of 20 or greater. The 24 1951 specimens and 35 1959 specimens indicate that the epidemiology of parainfluenza 3 in Tahiti did not change markedly between 1951 and 1959. There were 74% with titers of 20 or more in 1951 and 80% in 1959.

Seven out of 9 Baffinland sera (77%) had parainfluenza titers of 20 or greater.

Correlation with age. The titers are plotted against age in Figure 5. There are too few sera from the 2-8 year old group to describe accurately the onset of parainfluenza immunity. It might be noted, however, that among the Alaskan sera all the parainfluenza 3 titers of 20 or less occur in people who are less than 15 years old. Also, in general, after 12 years of age there are relatively more parainfluenza 3 titers of 40 or greater (75%) than in children under 12 (40%). This difference held in both Alaskan and Tahitian sera.

Comparison of mumps and parainfluenza 3 in isolated populations.

Figure 1 presents the distribution of antibody titers to mumps and parainfluenza 3 beside each other. It is clear that, in Alaska, Baffinland, and Tahiti, there are many low and few high mumps titers, while in the same populations there are few low and many high parainfluenza 3 titers.

Analysis of correlation between mumps and parainfluenza titers in Iceland.

Although titers of parainfluenza 1, 2, and 3 were run on 108

MUMPS TITERS	MUMPS HISTORIES: POSITIVE	MUMPS HISTORIES: NEGATIVE
HIGH (80 or MORE)	P.1 = 1.2 (log avj.) P.2 = 2.6 P.3 = 3.8 (17 IN GROUP)	P.1 = 1.2 P.2 = 2.7 P.3 = 3.2 (14)
MEDIUM (20, 40)	P.1 = 1.1 P.2 = 2.0 P.3 = 2.7 (20)	P.1 = 1.0 P.2 = 2.1 P.3 = 3.0 (22)
LOW (<20)	P.1 = 1.0 P.2 = 1.8 P.3 = 2.0 (12)	P.1 = 1.0 P.2 = 1.8 P.3 = 2.4 (21)

"P." = PARAINFLUENZA.
LOGS OF P. TITERS:

1 = 10
2 = 20
3 = 40
4 = 80
5 = 160
6 = 320

TABLE 2

of the Iceland sera, these data could not be used to describe the epidemiology of parainfluenza in Iceland because the sera were selected in a non-random fashion.

The sera were selected to provide a group with positive mumps histories, and an equal group with negative mumps histories. Then each of these groups was further subdivided into equal thirds having mumps titers of 80 or greater, titers of 20 or 40, and mumps titers 20. An attempt was made to fill the resultant six groups with similar numbers, similar average ages, and sera from a variety of geographical districts. Titers of parainfluenza 1, 2, and 3 were run.

On preliminary examination, regardless of the grouping just described, some correlations between mumps and parainfluenza were seen. For example, of 21 sera with mumps titers of 40 or more, 16 had high titers of parainfluenza 3 (>40). But of 34 sera with high parainfluenza 3 titers, only 12 had high titers of mumps.

Table 2 presents the geometric mean parainfluenza titers in sera grouped according to mumps history and titer. To obtain a geometric mean, logs to base 2 of parainfluenza titers were averaged, i.e., 1 for 10, 2 for 20, 3 for 40, 4 for 80, 5 for 160, 6 for 320.

In the case of each parainfluenza type, the titer correlated with the mumps titer. For example, in sera with a positive mumps history, the highest parainfluenza 3 average (3.8) was found in the group with the highest mumps titers (80 or greater); and the lowest parainfluenza 3 average (2.0) was found in the group with the lowest

mumps titers (<20). This trend was observed for each of the para-influenza viruses in all of the groups, whether mumps histories were positive or negative.

If corss reactions with parainfluenza were giving false mumps positive reactions one might expect these to occur where the homologous parainfluenza titers were highest. Since no difference was found between groups with different mumps histories it s eems unlikely that the mumps titers are attributable to parainfluenza experience.

The group with positive histories of mumps and low titers of mumps antibodies might be explained by mistaken diagnoses of similar diseases or decay of mumps antibodies. Table 2 shows that the parainfluenza titers in this group were lower than average, suggesting that parainfluenza viruses were not producing atypical "mumps" infections.

The parainfluenza titers were roughly similar in groups with similar mumps titers, whether the histories were positive or negative. Whatever influence mumps seemed to have on parainfluenza titers did not seem to be affected by the mumps history.

DISCUSSION

Definition of a "positive" mumps test.

The definition of "positive" or "negative" mumps serology depends on whether a titer of 20 is considered evidence of past infection or not. Among the isolated populations of Tahiti, Alaska, and Baffinland the definition makes little difference; there are

5% "positives" if 20 is not included, and only 15% if it is. But in Iceland, including titers of 20 raises the percentage of positives from 51 to 78. Most likely many of these titers of 20 are evidence of past infection. This seems especially probable in the light of the lowering of titers with age shown in Figure 2. For the purposes of this paper, it is sufficient to appreciate the low incidence of high mumps titers in the isolated populations, and the high incidence in Iceland.

Persistence of mumps antibodies.

Evidence for the persistence of mumps HI antibodies is the data presented in Figure 4 and the rather small group of Icelanders with positive histories and "negative" serologies (Figure 3). Bashe (18) has said that 30% of those with mumps histories did not have neutralizing antibodies. The similar group in this study represents 20 out of 114 with positive histories, or 17%. Figure 4 comprises a small number of cases, and the severity of these cases cannot be estimated, but it suggests a persistence of elevated titers for 20 years or more. The percentage of inapparent infections may present confirmatory evidence. If the percentage of inapparent infections were low, one might suppose that the lower titers produced by milder infections had died out. But the high average % of inapparent infections in Iceland suggests that HI antibodies persist reliably. HI antibodies as measured in this study seem to persist longer than neutralizing antibodies found by Bashe <u>et.al.</u> (6).

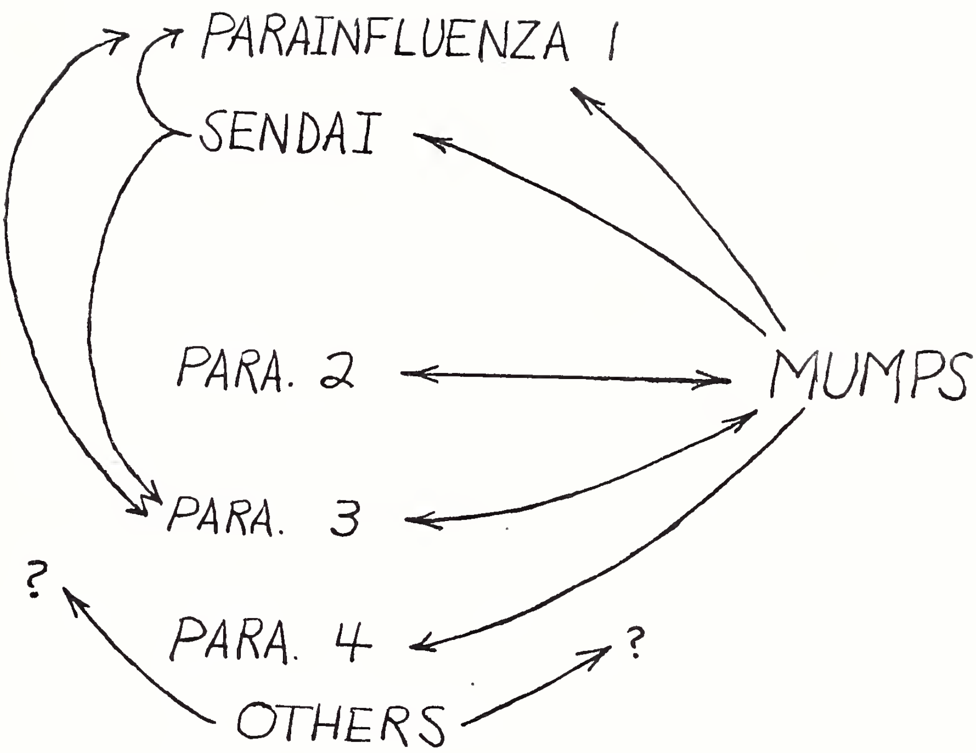


FIGURE 6

Cross reactions.

The literature. The cross reactions among the myxoviruses are a complicated subject. In Figure 6 the crosses reported in the literature are summarized (7,11,10,22,23,19,26,27,29). Arrows point from the immunizing agent to the agent against which cross reacting antibodies are formed.

Almost all of these reported cases were followed closely at the time of infection in the laboratory. In a number of cases (7,10,19,22,23) the immunizing agent was isolated. Double infections were not detected nor considered likely because of the frequency and range of cross reactions (10). In other cases (11), agents were not isolated, but primary immunizing agents were diagnosed by clinical pictures and serology. The serology in most reports consisted of complement fixation or HI tests. The HI tests were said to be most sensitive.

Parainfluenza producing false positive mumps serology. This paper addressed itself mainly to the cross reactions between the parainfluenza viruses and mumps; and in particular to false positive mumps serology due to prior parainfluenza infections. These false positive titers may have been looked on as "inapparent infections" or "inherent insusceptibility" in the old in earlier studies in which the parainfluenza-mumps relationship was not recognized. The evidence presented against the persistence of these cross reacting antibodies is of an epidemiological kind. First, Tahiti, Baffinland, and Alaska had considerable experience with parainfluenza 3, but few mumps antibodies. Second, in Iceland it was seen that positive para-

influenza 1, 2, and 3 serology followed positive mumps serology, and not the opposite. That is, individuals who had mumps antibodies were more likely to have high parainfluenza titers; but many who had parainfluenza antibodies did not have high mumps titers. Further there is the data presented in Table 2. If parainfluenza were producing false mumps positive reactions, the titers of parainfluenza antibodies would be higher in the group of negative mumps histories than in that of positive histories, especially in the group of high mumps antibody titer. But the parainfluenza titers were similar in groups of similar mumps titers, regardless of mumps history. In fact, all of the average parainfluenza titers seemed to follow mumps titers, and not the opposite.

"Inapparent infections" could have several meanings. At an intermediate level, 30-40% in the literature, it may refer only to what it says: subclinical mumps infection and positive serology. The fact that the incidence of inapparent infections remains at an elevated level through the upper age groups suggests that the antibody in these cases persists although the possibility that it is periodically boosted by a recent epidemic has not been eliminated. If the number of persons with no mumps history but positive serology were unusually high in some populations, it might suggest a high incidence of cross reactions due to prevalence of parainfluenza. In Iceland the number of inapparent infections was near the percentage reported in the literature.

This evidence suggests that parainfluenza viruses do not ofen

produce lasting mumps antibodies. That cross reactions frequently occur in this direction is incontrovertible.

It is possible that parainfluenza produces some degree of mumps immunity without producing detectable complement fixing or HI antibodies, which may have occurred in St. Lawrence. But, it seems most likely that measurable "mumps" antibodies stimulated by parainfluenza infection remain in the serum only a short time. Hsiung reported one case where the cross reacting antibody persisted for 3 months (10).

Mumps raising parainfluenza titers. There is some evidence in Table 2 that the opposite cross reaction in which mumps produces parainfluenza antibodies is a common occurrence. In every subgroup, each of the parainfluenza averages correlated with the mumps titer. For example, the parainfluenza 3 average was 3.8 in the group with high mumps titers, 2.7 in the medium range, and 2.0 in the group with low mumps titers. It must be remembered that each point in the log scale represents a twofold difference: 3.8, a titer of nearly 80, 2.0, a titer of 20. With good symmetry this difference is repeated in all groups. The isolated correlation might be explained by a cross reaction in either direction, or a common factor. But, the rest of the epidemiology of mumps in Iceland, and the examination of the group of inapparent infections, make parainfluenza-to-mumps crosses less likely; and no common factor raising both parainfluenza and mumps titers is known. This is epidemiological evidence confirming the evidence from small groups reported by Lennette and Chanock (11;7,22), that mumps raises parainfluenza titers.

<u>Partial immunity to mumps in adults</u>. The "inherent insusceptibility" of older persons described by Philip (13) remains unexplained. In the St. Lawrence Island mumps epidemic adults over 35 had the lowest attack rate, the lowest morbidity, the highest % of inapparent infections, and the highest antibody rises following clinical infections. This occurred despite the fact that prior to the epidemic adults showed no significant mumps complement fixing titers and there was no history of a mumps epidemic for the previous 50 years. The implication is that there is some degree of mumps immunity in older persons. In this paper there is no evidence that parainfluenza viruses often raise mumps HI antibodies. Possible explanations for the inherent insusceptibility of older persons remain: a "mumps" antibody other than a CF or HI antibody, an agent other than a parainfluenza virus producing the "mumps" antibody, or an unknown mechanism. The reliability of the HI test in diagnosis and its correlation with insusceptibility make it seem unlikely that it would detect <u>none</u> of this process. Any of the agents in Figure 6 or others, or a combination of agents, might play a part in increasing the immunity of older persons.

<u>Parainfluenza-caused mumps</u>.

It would be difficult to prove that a parainfluenza virus did not occasionally produce a clinical case of mumps, or a second case of mumps (12). If such a parainfluenza infection produced cross reacting mumps antibodies, diagnosing it would be much more difficult. In this paper, the group of Icelanders with positive mumps histories but negative mumps serologies might be explained by decay of mumps antibodies or parainfluenza-caused "mumps" infection. The first

explanation seems more likely for the following reasons. The percentage of positive mumps histories with negative serologies is rather low, 18% in Iceland. This seems within the limits of error of the HI technique. And secondly, in the group of Icelanders with positive mumps histories but negative serologies the parainfluenza titers are lower than in any other group. If parainfluenza were producing atypical "mumps", higher parainfluenza titers might have been expected. Again, this evidence is only suggestive that such atypical parainfluenza-caused cases of "mumps" do not occur often. Objections to the suggestion that parainfluenza might occasionally produce "mumps" are that diagnosis must be certain and Koch's postulates should be satisfied (25); but this is not covered in this paper.

SUMMARY AND CONCLUSIONS

1) In Alaska, Baffinland, and Tahiti, mumps antibodies were rare even though parainfluenza 3 antibodies were common.

2) Mumps antibodies were common in Iceland.

3) Mumps HI antibodies persist for 20 or more years.

4) The low incidence (18%) of positive mumps histories with low HI antibodies, the different epidemiology of mumps and parainfluenza 3 in the isolated populations, and the Iceland data in Table 2, make it unlikely that parainfluenza viruses often produce false positive mumps HI tests in normal populations.

6) The parainfluenza viruses probably do not often produce clinical "mumps".

7) Some immunizing effect of parainfluenza viruses against mumps, as suggested in the St. Lawrence epidemic, cannot be excluded.

Bibliography

1. Henle, Gertrude, Werner Henle, Jane S. Burgoon, Winslow Bashe, Jr., and Joseph Stokes, Jr. Studies on the Prevention of Mumps. The Determination of Susceptibility. Journal of Immunology, 66, p. 535, 1951.

2. Levens, Jeanette and John Enders. The Hemagglutinative Properties of Amniotic Fluid from Embryenated Eggs Infected with Mumps Virus. Science, 102, p. 117, 1945.

3. Aikawa, Jerry K., M.D., and Gordon Meiklejohn, M.D. The Serological Diagnosis of Mumps. Comparison of Three Methods. Journal of Immunology, 62, p. 261, 1949.

4. Robbins, Frederick, Lawrence Kilham, Jeannette Levens, and John Enders. An Evaluation of the Test for Antihemagglutination in the Diagnosis of Infections by the Mumps Virus. Journal of Immunology, 61, p. 235, 1949.

5. Enders, M.D., John. Techniques of Laboratory Diagnosis. Journal of Pediatrics, 29, p. 129, 1946.

6. Bashe, Winslow, Tamar Gottlieb, Gertrude Henle, and Werner Henle. Studies on the Prevention of Mumps. The Relationship of Neutralizing Antibodies to the Determination of Susceptibility and to the Evaluation of Immunization Procedures. Journal of Immunology, 71, p. 76, 1953.

7. Chanock, M.D., Robert, Robert Parrott, M.D., K. Cook, Ph D., B.E. Andrews, M.R.C.S., J. Bell, M.D., Thomas Reichelderfer, M.D., Albert Kapikion, M.D., F. Mastrota, M.D., and Robert Huebner, M.D. Newly Recognized Myxoviruses from Children with Respiratory Diseases. New England Journal of Medicine, 258, p. 207, 1958.

8. Andrewes, C.H., F.B. Bang, and F.M. Burnet. A Short Description of the Myxovirus Group. Virology, 1, p. 176, 1955.

9. Gottlieb, Tamar, Winslow Bashe, Jr., Gertrude Henle, and Werner Henle. Studieson the Prevention of Mumps. The Development of a Neutralization Test and its Application to Convalescent Sera. Journal of Immunology, 71, P. 66, 1953.

10. Hsiung, G.D., Peter Isaacsen, and Grace Tucker. Studies of the Parainfluenza Viruses: Studies on Serological Relations in Humans. The Yale Journal of Biology and Medicine, 35, p. 534, 1963.

11. Lennette, Edwin, Florence Jensen, Raymond Guenther, and Robert Magoffin. Serological Responses to Parainfluenza in Patients with Mumps Infections.
Journal of Laboratory and Clinical Medicine, 61, p. 780, 1963.

12. Bloom, H.H., K.M. Johnson, R. Jacobsen, and R.M. Chanock. Recovery of Parainfluenza Viruses from Adults with Upper Respiratory Infections. American Journal of Hygiene, 74, p. 50, 1961.

13. Philip, R.N., K.R. Reinhard, and D.B. Lackman. Observations on a Mumps Epidemic in a Virgin Population.
American Journal of Hygiene, 69-70, p. 91, 1959.

14. Horstman, Dorothy, and Lizbeth Kraft. Poliomyelitis and Other Antibody Pattern in Natives of Tahiti and Raiatea. Journal of Immunology, 75, p. 249, 1955.

15. Paul, J.R., and J.T. Riardan. Observation on Serological Epidemiology. Antibodies to the Lansing Strain of Poliomyelitis Virus in Sera from Alaskan Eskimos. American Journal of Hygiene, 52, p. 202, 1950.

16. Greenberg, PhD, L., and J.O. Blake, M.B. An Immunological Study of the Canadian Eskimo.
Canadian Medical Association Journal, 78, p. 27, 1958.

17. Rosen, Leon, and G. Thooris. Poliomyelitis in French Oceania. Epidemicological Observations on an Outbreak.
American Journal of Hygiene, 57. p. 237, 1953.

18. Salk, Jonas. A Simplified Procedure for Titrating the Hemagglutinating Capacity of Influenza Virus and the Corresponding Antibody.
Journal of Immunology, 49, p. 87, 1944.

19. Hsiung, G.D., Peter Isaacsen, and Robert McCallum. Studies of a Myxovirus Isolated from Human Beings. Isolation and Properties.
Journal of Immunology, 88, p. 284, 1962.

20. Public Health in Iceland. Reykjavik. Years 1940 through 1957.

21. Van Euler, Leo, Fred. S. Kantor, and G.D. Hsiung. Studies of Parainfluenza Viruses. Clinical, Pathological, and Virological Observations. The Yale Journal of Biology and Medicine, 35, p. 523, 1963.

22. Chanock, M.D., Robert M. Association of a New Type of Cytopathogenic Myxovirus with Infantile Croup.
Journal of Experimental Medicine, 104, p. 555, 1956.

23. Chanock, Robert, M.D., Doris Wong B.A., Robert Huebner, M.D., and Joseph Bel, M.D. Serological Responses of Individuals Infected with Parainfluenza Viruses.
American Journal of Public Health, 50, p. 1858, 1960.

24. La Placa, M., and C. Moscovici. Distribution of Parainfluenza Antibodies in Different Populations. Journal of Immunology, 88, p. 72, 1962.

25. Johnsen, M.D., Claud, and Ernest Goodpasture, M.D. An Investigation of the Etiology of Mumps.
Journal of Experimental Medicine, 59, p. 1, 1934.
American Journal of Hygiene, 21, p. 46, 1935.

26. Johnsen, Karl, Robert Chanock, M.K. Cook, and Robert Huebner. Studiesof a New Hemagglutinating Virus. Isolation Properties, and Characterization.
American Journal of Hygiene, 71, p. 81, 1960.

27. De Meio, Joseph and Duard L. Walker. Demonstration of an Antijen Relationship between Mumps Virus and the Hemmagglutinating Virus of Japan. Journal of Immunology, 78, p.465, 1957.

28. Maris, Elizabeth, M.D., John Enders, M.D., Joseph Stokes, Jr., M.D., and Lewis Kane, M.D. Immunity in Mumps. The Correlation of the Presence of Complement-fixing Antibodies and Resistence.
Journal of Experimental Medicine, 84, p. 323, 1946.

29. Jordan, M.D., William, and A.E. Feller, M.D. The Relation of Complement-fixing and Antihemagglutinating Factors Versus of Mumps and Newcastle Disease.
Journal of Laboratory and Clinical Medicine, 35, p. 369, 1950.

30. Black, F.L., and Margret Dudnadottir, Measles Vaccination of Adults in Iceland.
Lancet, p. 418, February 23, 1963.

3. Gudnadottir, Margret, and F.L. Black, Ph.D., Response of Adults in Iceland to Live Attenuated Measles Vaccine. To be Published.